

ORIGINAL ARTICLE

Q1 Different displacement of bioimpedance vector due to Ag/AgCl electrode effect

L Nescolarde¹, H Lukaski², A De Lorenzo³, B de-Mateo-Silleras⁴, MP Redondo-del-Río⁴ and MA Camina-Martín⁴

Q2

BACKGROUND/OBJECTIVES: Bioelectrical impedance vector analysis (BIVA) is increasingly used in clinical research to assess soft tissue hydration. It is known that physical characteristics of electrodes, such as low intrinsic impedance, low electrode/skin contact impedance and type of gel, affect the reliability of noninvasive bioimpedance assessments. The aim of this study was to determine the effect of intrinsic impedance of electrode on the bioimpedance vector displacement in RXc graph.

SUBJECTS/METHODS: The intrinsic impedance is measured in nine pregelled disposable Ag/AgCl electrodes usually used for bioimpedance measures. The BIVA method is performed on 35 healthy volunteers using a 50 kHz phase-sensitive bioimpedance analyzer (BIA 101 Anniversary) with the lowest intrinsic impedance electrode and highest. The individual bioimpedance vector is plotted on the bivariate normal interval of reference population. The differences in the mean bioimpedance vectors obtained with each electrode are plotted, with their 95% confidence ellipses, on the dRXc graph. The paired one-sample Hotelling's T^2 -test is used to compare the differences of the mean bioimpedance vectors.

RESULTS: We found large variability in intrinsic resistance (11–665 Ω) and reactance (0.25–2.5 Ω) values of the electrodes analyzed and significant displacement ($P < 0.05$) of bioimpedance vector positions in healthy adults according to the paired one-sample Hotelling's T^2 -test.

CONCLUSIONS: A robust study of all physical characteristics of commercial Ag/AgCl electrodes is necessary to reach consensus on pregelled Ag/AgCl electrodes valid for bioimpedance measurement. This information will enable BIVA users to avoid systemic errors when performing BIVA assessments, specifically when these measurements are used for clinical interpretations.

European Journal of Clinical Nutrition (2016) **00**, 1–7. doi:10.1038/ejcn.2016.121

INTRODUCTION

Q3

The use of bioelectrical impedance methods to assess the structure and function of biological entities continues to increase at a rapid pace.¹ These noninvasive methods use modern electronic devices to introduce a safe alternating current into the body at single or multiple frequencies and to measure passive bioelectrical properties that are related to physiological parameters or body composition. A key component of bioimpedance methods is the use of contact surface electrodes, although its use is not universal. Indeed, the use of steel contact electrodes as used in stand-on-type devices is increasing.

Despite the plethora of research published using these methods, a conspicuous gap exists in standardization of the electrodes required to measure bioelectrical variables *in vivo*. Guidelines from the NIH Technology Assessment Conference on Bioelectrical Impedance Analysis,² a subsequent follow-up review,³ and the European Society for Parenteral and Enteral Nutrition⁴ only address the size of the electrodes and separation between source and detector electrodes for tetrapolar measurements. The topic of characteristics of the contact electrodes is not fully addressed.

Surface electrodes composed of silver-silver chloride (Ag/AgCl) are a common component of bioimpedance systems that are routinely used in a wide range of biomedical applications. The scientific basis for the use of Ag/AgCl surface electrodes is well

established for noninvasive assessment of bioelectrical signals.^{5–10} Webster¹¹ emphasized that theoretically every Ag/AgCl electrode should have the same intrinsic impedance half-cell potential, but he also anticipated that differences probably exist among Ag/AgCl electrodes and it can introduce errors into bioelectrical measurements.

Concern about variability of intrinsic Z among commercial Ag/AgCl electrodes persists. Shiwei *et al.*¹² found disparity in the measured impedance values among the same volunteers with different pregelled Ag/AgCl commercial electrodes. Emerging evidence indicates the need to evaluate commercial Ag/AgCl electrodes for bioimpedance applications.

Some researchers recommend the use of contact Ag/AgCl electrodes with appropriate characteristics (example, low intrinsic Z and low electrode/skin contact impedance, type of gel), careful placement of the electrodes and avoidance of expired electrodes to minimize measurement errors.^{8–10,13,14} The electrode/skin contact impedance depends on the type and preparation of the skin and the time the electrode has been placed. Usually, it is a measure using the three-electrode configuration.¹⁰

There are different methods for assessment of body composition by noninvasive measures of bioelectrical impedance. An alternative impedance approach is the utilization of the spatial relationships between resistance (R) and reactance (X_c) to assess soft tissue hydration by RXc graph, called the bioelectrical

¹Department of Electronic Engineering, Universitat Politècnica de Catalunya, Barcelona, Spain; ²Department of Kinesiology and Public Health Education, Hyslop Sports Center, University of North Dakota, Grand Forks, ND, USA; ³Department of Neuroscience, Division of Human Nutrition, University of Rome Tor Vergata, Rome, Italy and ⁴Area of Nutrition and Food Science, Faculty of Medicine, Universidad de Valladolid, Valladolid, Spain. Correspondence: Dr L Nescolarde, Department of Electronic Engineering, Universitat Politècnica de Catalunya, C/Jordi Girona 1-3, Barcelona 08034 Spain.

E-mail: lex.nescolarde@upc.edu

Received 19 April 2016; revised 23 May 2016; accepted 7 June 2016

impedance vector analysis (BIVA) method,¹⁵ which has become one of the most used in clinical applications in the last 20 years.

The BIVA method has been validated in numerous clinical research such as hemodialysis,^{16–18} peritoneal dialysis,^{19,20} obesity,²¹ heart failure,²² anorexia nervosa,²³ pediatrics,^{24–28} healthy adults,^{28–31} geriatrics^{32–34} or lung cancer.³⁵ The main advantage of this method is the lack of reliance on any assumption about constant soft tissue hydration and chemical composition of the fat-free body, and its independence from the use of body weight for any assessment. Thus, BIVA only depends on the reproducibility and accuracy of bioimpedance measurements and intraindividual variability in body composition. Other advantages of the method are its simplicity and objectivity.³⁶

The objective of this research was to determine the effect of intrinsic impedance of pregelled Ag/AgCl electrodes on displacement of impedance vectors using the BIVA method on healthy human volunteers. We tested the hypothesis that differences in intrinsic electrode impedance affect the displacement of impedance vectors, determined by the BIVA method, used in clinical assessments.

MATERIALS AND METHODS

Measurement protocol in volunteers

This was a cross-sectional study performed on a convenience sample of 35 Italian healthy volunteers aged 30–50 years. Potential volunteers were excluded if they had any underlying medical conditions or current infections, if they were taking any drugs that could influence bioimpedance measurements and if they had physical abnormalities (amputations and so on). All measurements were performed on a single day by trained staff according to standardized protocols. Informed consent was obtained from all subjects, and the Ethics Committee 'Comitato Etico Indipendente c/o Fondazione PTV Policlinico Tor Vergata' approved the study.

BIVA of tetrapolar whole-body measure was performed on 35 healthy volunteers: 15 males (age: 30–50 years; height: 1.77 (0.05) m; weight: 76.6 (13.8) kg; body mass index (BMI) 24.7 (3.9) kg/m²) and 20 females (age: 30–50 years; height: 1.65 (0.03) m; weight: 57.3 (4.0) kg; BMI 21.0 (1.9) kg/m²) who underwent standard whole-body tetrapolar bioimpedance measurements. The volunteers were measured in a supine decubitus position on a non-conductive litter in the biomedical laboratory (22–24 °C). Two electrodes injecting the current (*I*) and two electrodes detecting voltage (*V*) and phase were dorsally placed on the right hand in the third metacarpus-phalangeal articulation and in the carpus, respectively. The pair on the foot was located in the third metatarsus-phalangeal and in the articulation.³⁷

The bioimpedance measurements were measured using two commercial Ag/AgCl electrodes with different intrinsic impedance. The order of

electrode use was randomized for each volunteer. Technical staff used cotton swabs soaked with alcohol to cleanse the skin before each electrode application. Measurements were performed 10 min after electrode placement, which is grounded on the recommendation of Bolton *et al.*¹³

A calibrated scale (SECA 700 Mechanical Scale; Seca Corp., Hamburg, Germany) and wall-mounted stature meter (SECA 240; Seca Corp.) were used to measure body weight and standing height, respectively. **Q4**

The measurements were performed with a bioimpedance analyzer (BIA 101 Anniversary; AKERN-Srl, Florence, Italy) that applied a constant current of 240 μ A_{RMS} at 50 kHz, sine, phase-sensitive impedance instrument with a range of measure for *R*=0–1500 Ω and *Xc* 0–500 Ω . Tolerance was 2% max.

Intrinsic impedance measure of commercial Ag/AgCl electrodes

The intrinsic impedance variables (*R*, *Xc* and *Z*) were measured using commercial Ag/AgCl electrodes from six different manufacturers. Characteristics of the electrodes (contact surface area, shape, snap or tap electrode) are shown in Table 1. All electrodes were used within 1 year before the expiration date.

Figure 1 shows the arrangement of electrodes and the dual layer of matched electrodes to determine the intrinsic impedance for each pair of the nine Ag/AgCl electrodes analyzed.

A 50 kHz, phase-sensitive device (BIA 101 Anniversary) with a tetrapolar electrode method introduced a sinusoidal AC of 240 μ A_{RMS} and measured *R*, *Xc* and phase angle (PhA). Calibration of the system was confirmed with

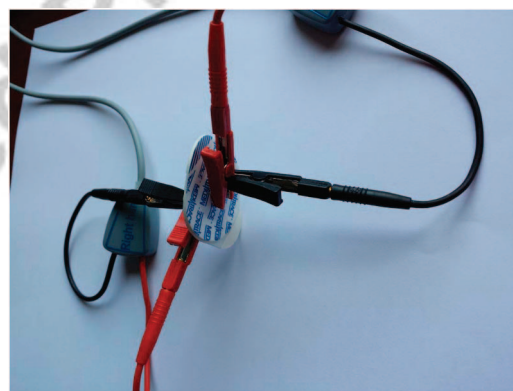


Figure 1. Electrode wafer to measure intrinsic impedance with a phase-sensitive 50 kHz bioimpedance device. Red clips inject current and black clips sense voltage.

Table 1. Characteristics of the nine commercial electrodes used in bioimpedance measurements

Manufacturer	Reference of electrode	Conductive surface area (cm ²)	Snap or tab electrode	Shape
COVIDIEN-Kendall	5500 Diagnostic tab electrodes/30807732	5.75 cm ² (2.3 × 2.5 cm ²)	Tab	Rectangular
	200 Foam electrodes/31050522	4.40 cm ²	Snap	Circular
AKERN-Srl	BIATRODES/OELB	7.83 cm ² (2.7 × 2.9 cm ²)	Tab	Rectangular
ImpediMed	IU0GELTD	5.75 cm ² (2.3 × 2.5 cm ²)	Tab	Rectangular
3M	Red Dot/2239	2.45 cm ²	Snap	Circular
	Red Dot/2228	13.2 cm ² (4.0 × 3.3 cm ²)	Snap	Rectangular
Ambu	WS-00-S	8.57 cm ²	Snap	Circular
	SU-00-C	7.72 cm ²	Snap	Circular
ConMed	Softrace Large/2350	5.75 cm ² (3.2 × 3.2 cm ²)	Snap	Square

a precision parallel circuit formed by a 384 Ω ($\pm 1\%$) resistor parallel with 780 pF ($\pm 2\%$) capacitor.

Measurements of the intrinsic impedance of the electrodes (that is, R , X_c and Z) were performed on five pairs of each type of commercial electrodes of the same batch to check the intraelectrode variability of the intrinsic impedance values for each type of the commercial electrodes used.

BIVA method

The BIVA method is based on the measurement of the complex electrical impedance in whole-body (right-side) configuration. The components of the impedance vector, R and X_c , are normalized by the height of the subjects (R/H and X_c/H) and represented in the RX_c graph (abscissa R/H , ordinate X_c/H). The vector measured in an individual is compared against the normal interval of the reference population, as expressed in percentiles of 50, 75 and 95% of the normal distribution (Gaussian) bivariate, the probabilistic graph. The correlation between R and X_c determines the ellipsoidal form of the bivariate probability distributions. Variations in the structure of soft tissue are associated with a migration of the vector in the smaller axis (X_c/H) direction of the ellipses, with either an increase or a decrease in the PhA, whereas combined variations of hydration and nutrition are associated with migration of the vector in the direction of the combination of the two principal directions.¹⁵

In this study, the individual bioimpedance vectors obtained with each electrode were plotted on the tolerance ellipses (RX_c graph) of the Caucasian ethnicity adult population³² by using the BIVA software.³⁸ The 95% confidence ellipses for mean vectors of the measurements performed with each electrode were drawn to assess the effect of the intrinsic impedance of the electrodes on bioimpedance measurements.

Statistical analysis

The differences in the mean bioimpedance vectors obtained with each electrode were plotted, with their 95% confidence ellipses, on the dRX_c graph. The paired one-sample Hotelling's T^2 -test,³⁹ a multivariate extension of the Student's t -test for paired data, was used to compare the differences of the mean bioimpedance vectors. If a 95% confidence ellipse of a vector displacement covers the null vector (that is, abscissa 0, ordinate 0), the vector displacement is not statistically significant ($P < 0.05$) at a paired one-sample Hotelling's T^2 -test.³⁸

Student's t -test for paired data was used to compare the bioelectrical parameters (that is, R , X_c , R/H , X_c/H and PhA) measured with the electrodes of different intrinsic R and X_c for each subject, and repeated-measures analysis of variance was applied to assess differences in the five measurements of the intrinsic R , X_c and impedance values performed for each type of electrodes. Finally, statistical analysis of the interelectrode comparisons was performed using one-way analysis of variance. Statistical analysis was carried out using the SPSS version 19.0 (SPSS, Chicago, IL, USA). It was performed by an independent statistician, blinded for the reference numbers of the electrodes. All data are presented as mean (s.d.). The normality of the distribution of the variables was checked by the Shapiro–Wilk test and the homogeneity of variances by Levene's test.

RESULTS

Table 2 shows the measurements of the intrinsic R , X_c and Z values described as mean (s.d.), measured at 50 kHz, for each pair of the nine electrodes analyzed with the dual-layer wafer method. No significant differences were observed in the repeated measurements of the intrinsic R , X_c and Z values for each pair of the electrodes of the same type. However, the variability of the intrinsic R and X_c between the different types of electrodes was ~ 11 –665 and 0.25–2.5 Ω , respectively. The intrinsic R , X_c and Z values were statistically different between all the electrodes analyzed, except (1) R and Z values for Ambu/WS-00-S and ImpediMed-IU0GELTD electrodes and (2) X_c values for Ambu/SU-00-C and COVIDIEN-Kendall/30807732 electrodes, and X_c values for ImpediMed-IU0GELTD and 3M Red Dot/2239 electrodes.

Table 3 shows R and X_c values obtained on whole-body configuration, with electrode COVIDIEN-Kendall/31050522 and with electrode Ambu/SU-00-C in 35 healthy volunteers. R/H and X_c/H , PhA and linear coefficient correlation r (R , X_c) are also shown in Table 3. The mean values of the bioelectrical parameters of the volunteers measured with the electrodes that showed the highest intrinsic impedance (Ambu/SU-00-C) were significantly higher compared with those obtained with the electrodes that showed the lowest intrinsic impedance (COVIDIEN-Kendall/31050522). Specifically, the PhA values obtained with the electrode COVIDIEN-Kendall/31050522 were $\sim 1^\circ$ lower compared with those obtained with the electrode Ambu/SU-00-C.

Figure 2 shows the individual bioimpedance vectors for 15 male and 20 female healthy volunteers, obtained with the BIVA software,³⁸ on the bivariate normal interval of reference population³⁵ separated by gender and determined with the electrodes, which had the lowest and the highest intrinsic impedance (COVIDIEN-Kendall/31050522 and Ambu/SU-00-C, respectively).

Figure 3 shows the 95% confidence ellipses corresponding to COVIDIEN-Kendall/31050522 and Ambu/SU-00-C plotted on the bivariate normal interval of reference population.³⁵ The paired graph shows the 95% confidence ellipse corresponding to the difference in the mean bioimpedance vector obtained with the electrodes COVIDIEN-Kendall/31050522 and Ambu/SU-00-C (Figure 4) for males (blue line) and females (red line). The 95% confidence ellipse of the vector displacement does not cover the null vector (that is, $dZ/H = 0.0$), indicating a statistically significant vector displacement ($P < 0.05$) at a paired one-sample Hotelling's T^2 -test.³⁸

Table 2. R , X_c and Z intrinsic values determined, at 50 kHz, for the nine commercial electrodes used in bioimpedance measurements

Electrode/reference	R (Ω)	X_c (Ω)	Z (Ω)
COVIDIEN-Kendall/30807732	347.19 (0.04) ^{a,b,c,d,e,f,g,h}	2.50 (0.00) ^{a,b,c,d,e,f,h}	347.20 (0.04) ^{a,b,c,d,e,f,g,h}
COVIDIEN-Kendall/31050522	10.89 (0.04) ^{b,c,d,e,f,g,h}	0.30 (0.00) ^{b,c,d,e,f,g,h}	10.89 (0.04) ^{b,c,d,e,f,g,h}
AKERN-BIATRODES/OELB	25.74 (0.04) ^{c,d,e,f,g,h}	0.45 (0.00) ^{c,d,e,f,g,h}	25.74 (0.04) ^{c,d,e,f,g,h}
ImpediMed-IU0GELTD	27.13 (0.05) ^{d,e,g,h}	0.90 (0.00) ^{e,f,g,h}	27.14 (0.05) ^{d,e,g,h}
3M Red Dot/2239	15.75 (0.04) ^{e,f,g,h}	0.90 (0.00) ^{e,f,g,h}	15.77 (0.04) ^{e,f,g,h}
3M Red Dot/2228	24.75 (0.04) ^{f,g,h}	0.70 (0.00) ^{f,g,h}	24.76 (0.04) ^{f,g,h}
Ambu/WS-00-S	27.24 (0.04) ^{g,h}	0.25 (0.00) ^g	27.24 (0.05) ^{g,h}
Ambu/SU-00-C	665.37 (0.04) ^h	2.50 (0.00) ^h	665.37 (0.04) ^h
ConMed/2350	30.23 (0.04)	0.25 (0.00)	30.23 (0.04)

Results are described as mean (s.d.). Abbreviations: R , resistance; X_c , reactance; Z , impedance. ^aMean values were significantly different from those of the COVIDIEN-Kendall/31050522 ($P < 0.05$). ^bMean values were significantly different from those of the AKERN-BIATRODES/OELB ($P < 0.05$). ^cMean values were significantly different from those of the ImpediMed-IU0GELTD ($P < 0.05$). ^dMean values were significantly different from those of the 3M Red Dot/2239 ($P < 0.05$). ^eMean values were significantly different from those of the 3M Red Dot/2228 ($P < 0.05$). ^fMean values were significantly different from those of the Ambu/WS-00-S ($P < 0.05$). ^gMean values were significantly different from those of the Ambu/SU-00-C ($P < 0.05$). ^hMean values were significantly different from those of the ConMed/2350 ($P < 0.05$).

Table 3. Bioelectrical parameters determined, at 50 kHz, for the two commercial electrodes used for bioimpedance measurements in the volunteers

	Males (n = 15)		Females (n = 20)	
	COVIDIEN-Kendall/31050522	Ambu/SU-00-C	COVIDIEN-Kendall/31050522	Ambu/SU-00-C
<i>R</i> (Ω)	466.2 (89.0) ^a	470.7 (86.6)	572.8 (67.2)	574.7 (69.6)
<i>Xc</i> (Ω)	55.8 (9.6) ^a	64.2 (9.3)	65.6 (9.5) ^a	75.0 (8.7)
<i>Z</i> (Ω)	469.6 (89.1) ^a	475.1 (86.6)	575.9 (67.5)	579.6 (69.8)
<i>R/H</i> (Ω/m)	254.5 (50.7)	266.2 (51.5)	343.3 (36.4)	347.3 (40.6)
<i>Xc/H</i> (Ω/m)	31.0 (5.7) ^a	36.3 (5.5)	38.9 (5.1) ^a	45.4 (5.4)
<i>Z/H</i> (Ω/m)	265.6 (52.9) ^a	268.7 (51.5)	348.0 (39.2)	350.3 (40.8)
PhA (deg.)	7.0 (1.1) ^a	7.9 (1.0)	6.5 (0.7) ^a	7.5 (0.7)

Abbreviations: COVIDIEN-Kendall/31050522, electrodes with the lowest intrinsic impedance; Ambu/SU-00-C, electrodes with the highest intrinsic impedance; *R/H*, resistance normalized by height; *Xc/H*, reactance normalized by height; *Z/H*, impedance normalized by height; PhA, phase angle. Results are described as mean (s.d.). ^aMean values were significantly different from those of the Ambu/SU-00-C ($P < 0.05$).

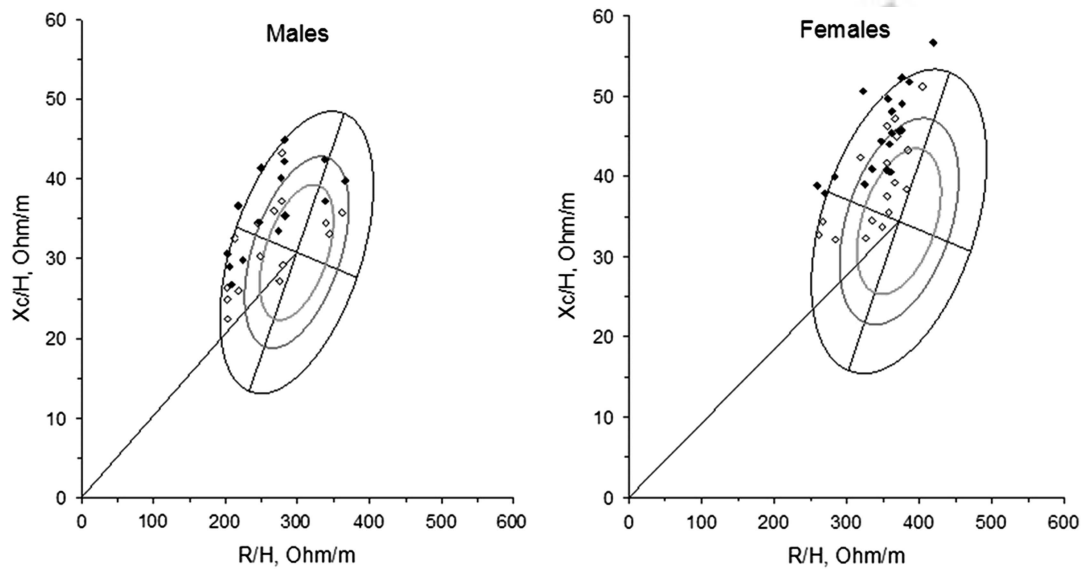


Figure 2. Individual vectors for 35 healthy volunteers (15 males and 20 females) on the tolerance ellipses (50, 75 and 95% percentiles) for the reference population. White squares, electrode with the lowest intrinsic impedance (COVIDIEN-Kendall/31050522); black squares, electrode with the highest intrinsic impedance (Ambu/SU-00-C).

DISCUSSION

The use of bioimpedance methods for clinical research has advanced in a deliberate manner. Early applications focused on the estimation of body composition by using several prediction equations.⁴ These approaches, however, have several limitations due to the technical and biological errors related to the assumptions of the constancy of the chemical composition of the fat-free body, as well as to the need to use appropriated cross-validated predictive equations developed in populations with similar biological and clinical characteristics to guarantee both the accuracy and the precision of the estimates when applying these equations in other populations (that is, their external validity).^{1,40} Current approaches emphasize the use of raw whole-body bioimpedance measurements such as the BIVA method,¹⁵ rather than derived variables to assess physiological, functional and nutritional status of individuals.^{33,36,41} The main advantage of this method is the avoidance of any assumption about constant soft tissue hydration and its independence from reliance on body weight as a predictor in regression prediction equations. This approach only depends on the reproducibility and accuracy of bioimpedance measurements and intraindividual variability in body composition, as well as its simplicity and objectivity.³⁶

Physical aspects for Ag/AgCl electrodes such as low intrinsic impedance, low electrode/skin contact impedance and the type of gel, among others, minimize the measurement errors.^{8–10,12–14}

Other considerations related to the electrode placement, such as careful placement and positioning of the electrodes, and avoidance of expired electrodes, also contribute to minimize measurement errors.^{10,13,14} Ideally, every Ag/AgCl electrode should have the same intrinsic impedance;¹¹ nevertheless, as has been observed in this study (Table 1), substantial differences do exist between commercial electrodes (Table 2).

Theoretically, the ideal electrode should have negligible intrinsic impedance;¹¹ therefore, electrodes with the lowest intrinsic impedance are the most appropriate to perform bioimpedance measurements in humans.¹¹ According to Boone and Holder,⁴² electrodes may be grouped according to the measured intrinsic impedance and relative to the impedance of unprepared skin, whose values at 50 kHz are between 100 and 200 Ω .

In this study, using 50 kHz, phase-sensitive device (BIA 101 Anniversary; AKERN-Srl) with a tetrapolar method and a sinusoidal AC of 240 μ ARMS, we measured the intrinsic value of *R* and *Xc*, of nine commercial Ag/AgCl electrodes selected (Table 1). As can be

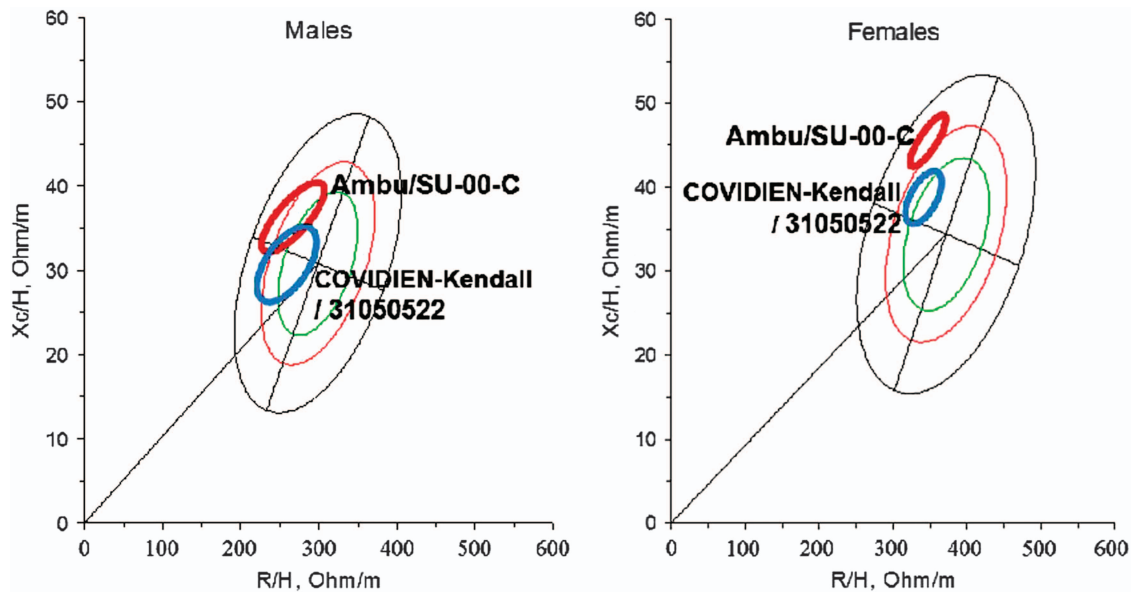


Figure 3. The 95% confidence ellipses for males and females plotted on the bivariate normal interval of reference population. COVIDIEN-Kendall/31050522 (blue line), electrodes with the lowest intrinsic impedance; Ambu/SU-00-C (red line), electrodes with the highest intrinsic impedance.

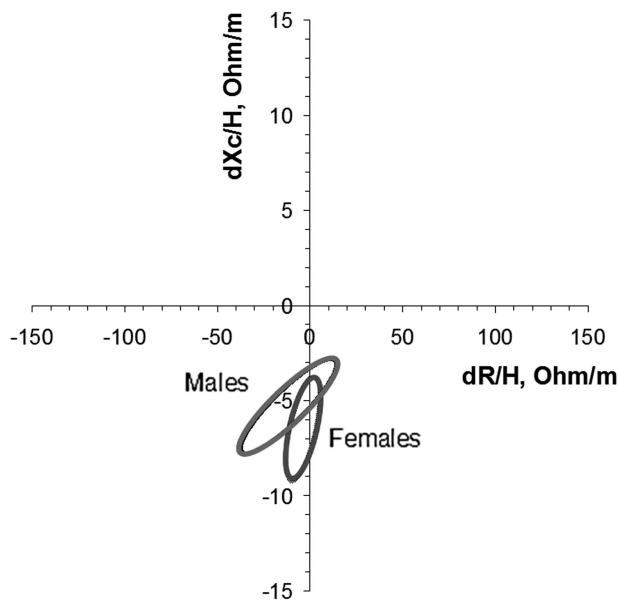


Figure 4. Paired graph for males and females. The paired graph shows the 95% confidence ellipse corresponding to the difference in the mean bioimpedance vector obtained with the electrodes COVIDIEN-Kendall/31050522 and Ambu/SU-00-C. Blue line, males; red line, females.

observed in Table 2, seven of the nine electrodes studied showed values $< 100 \Omega$ ($11 \pm 30 \Omega$), being all valid for noninvasive bioimpedance measurement at 50 kHz. Only two electrodes showed values $> 200 \Omega$ ($347 \pm 665 \Omega$).

The intrinsic impedance, measured with the tetrapolar method, is easy to measure by users (Figure 1). Four-electrode (tetrapolar) technique removes the contact impedance with respect to the two-electrode technique. In addition, it is less sensible to skin contact impedance mismatch (differences on the contact of the electrode's electrolyte with the skin), the status of the skin

(abraded or not), time passed between the placement of the electrodes and the type of electrolyte used for the electrode. As skin contact impedance is unknown, when using the two-electrode technique produces great errors in the impedance measurement.¹⁴ The effects of mismatch on contact impedance on the bioimpedance measurements with two commercial bioimpedance analyzer were tested in Bogónez-Franco *et al.*¹⁴

Given the large variability observed in intrinsic impedance (11–665 Ω), we used the lowest and the highest intrinsic impedance (that is, COVIDIEN-Kendall/31050522 and Ambu/SU-00-C, respectively) to analyze the effect of the intrinsic impedance of the electrodes on BIVA results in 35 healthy volunteers.

As can be seen in Figure 2, the individual vectors derived from bioimpedance measurements with the low intrinsic impedance electrode (COVIDIEN-Kendall/31050522) are within the 50% tolerance ellipse of the bivariate normal interval of reference population.³⁵ With increasing intrinsic impedance of electrodes (Ambu/SU-00-C), the vectors migrate ($P < 0.05$) into the upper left quadrant of the RXc graph and into the 75% tolerance ellipse. This displacement indicates a change in hydration classification from dehydration toward dehydration.³⁶ Figure 3 is consistent with Figure 2, given that the mean impedance vectors obtained with COVIDIEN-Kendall/31050522 for both males and females lie closest to the 50% tolerance ellipse of the reference population,³⁵ whereas the mean impedance vectors obtained with Ambu/SU-00-C lie between 75 and 95% percentiles. This finding of vector displacement in the same individuals, besides being statistically significant according to the paired one-sample Hotelling's T^2 -test (Figure 4), can have adverse effects on clinical interpretation of bioimpedance measurements.

To our knowledge, this is the first study focused on analyzing the effects of the intrinsic impedance of commercial Ag/AgCl electrodes on displacement of impedance vectors using the BIVA method; hence, we cannot compare our results with other studies. Nevertheless, our findings indicate a need to clarify not only the measurement protocol and the characteristics of the impedance analyzer but also the characteristics of the electrodes used for bioimpedance measurements when analyzing body composition of hydration status. In this regard, it should be noted that currently most of the studies in the field of body composition analysis

focused on the BIVA method do not provide information about the characteristics of the electrodes. This is a major problem as it makes it difficult, or even impossible, to compare databases obtained from different reference populations if measurements are performed with electrodes with different intrinsic impedance values.

On the other hand, recent studies have evidenced that PhA is an independent prognostic marker in ill patients and is associated with mortality, nutritional and functional status, and with health-related quality of life.^{43–45} PhA reference percentiles have been published as a simple and prognostically cutoff for detection of patients with cancer.⁴⁶ In this regard, we also found substantial differences in PhA values, $\sim 1^\circ$, depending on the electrodes used. This reveals the need to provide the characteristics of the commercial electrodes (that is, intrinsic impedance, electrode/skin contact impedance, and so on) together with the BIAs and the measurement protocol when publishing reference data, as well as the need to use similar electrodes when using bioelectrical measurements in both clinical and epidemiological settings.

However, it should be noted that manufacturers of BIAs recommend specific electrodes and that researchers should ideally use the electrodes recommended by the manufacturers (example, BIATRODES for AKERN, IUOGELTD for ImpediMed, Bodystat electrode for BodyStat analyzers and so on) and consult the data sheets for the electrodes provided by the manufacturers. Nevertheless, actually most of the studies in the field of body composition analysis use electrodes that have not been designed specifically for bioimpedance measurements (example, electrodes for electrocardiogram) and these electrodes can be valid for bioimpedance measurements, provided that their intrinsic impedance is low. For these reasons, we consider that it is necessary that researchers who work in the field of body composition analysis with bioimpedance techniques incorporate the characteristics of the intrinsic impedance of the Ag/AgCl measured at the standard frequency for body composition analysis (that is, 50 kHz) and with a phase-sensitive BIA. Similarly, when comparing databases of body composition measurements obtained from different populations, it is necessary to guarantee that measurements are performed with the same protocol, and the same BIA and electrodes to reduce any potential sources of error.

Finally, it is also worth emphasizing that bioelectrical impedance analysis methods are accurate for body composition analysis both in clinical setting and in large epidemiological studies. Bioimpedance measurements are simple, repetitive, noninvasive and show a minimal intra- and interobserver variability.⁴⁷ In addition, the body composition analysis based on bioimpedance techniques requires inexpensive and portable equipment and thus is a good alternative to other methods such as dual X-ray absorptiometry or magnetic resonance imaging, which are more expensive and not feasible to conduct in the field studies. The results of this study indicate that the intrinsic impedance of the electrodes produces a displacement of impedance vector using the BIVA method; hence, this effect must be taken into consideration when performing bioimpedance measurements to minimize potential bias. However, this is not the reason for assuming that bioimpedance measurements are not accurate.

To summarize, a robust study of all physical characteristics of commercial Ag/AgCl electrodes commonly used in bioimpedance measures is necessary owing to the influence on interpretation on the BIVA method. The consensus in Ag/AgCl electrode use in bioimpedance measurement will allow researchers to compare and discuss more consistently the results.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank all volunteers for their collaboration in this work without which it would not have been possible. We also thank Dr Eng Paco Bogónez-Franco for all recommendations and advice.

REFERENCES

- Lukaski HC. Evolution of bioimpedance: journey from assessment of physiological function through body fat to clinical medicine. *Eur J Clin Nutr* 2013; **67**: S2–S9.
- National Institutes of Health. Bioelectrical impedance analysis in body composition measurement. NIH Technical Assessment Statement 1994. *Am J Clin Nutr* 1996; **64**(Suppl): S245–S325.
- Ellis KJ, Bell SJ, Chertow GM, Chumlea WC, Know TA, Kotler DP et al. Bioelectrical impedance methods in clinical research: a follow up to the NIH technology assessment conference. *Nutrition* 1999; **15**: 874–880.
- Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gomez JM et al. Bioelectrical impedance analysis—part II: utilization in clinical practice. *Clin Nutr* 2004; **23**: 1430–1453.
- Schwan HP. Alternating current electrode polarisation. *Biophysik* 1966; **3**: 181–201.
- Janz GJ, Ives DJG. Silver-silver chloride electrodes. *Ann NY Acad Sci* 1968; **148**: 210–221.
- Crenner F, Angel F, Ringwald C. Ag/AgCl electrode assembly for thin smooth muscle electromyography. *Med Biol Eng Comput* 1989; **27**: 346–356.
- McAdams ET, Henry P, Anderson JM, Jossinet J. Optimal electrolytic chloriding of silver ink electrodes for use in electrical impedance tomography. *Clin Phys Physiol Mes* 1992; **13**(Suppl 1): 19–23.
- McAdams ET, McLaughlin J, Brown BH, McArdle F. The NIBEC E.I.T. electrode harness. In: Holder D (ed), *Clinical and Physical Applications of Electrical Impedance Tomography*. UCL Press: London, UK, 1993, pp 85–92.
- McAdams ET, Lackermeier A, McLaughlin JA, Macken D. The linear and non-linear electrical properties of the electrode-electrolyte interface. *Biosens Bioelectron* 1995; **10**: 67–74.
- Webster JG. *Medical Instrumentation, Application and Design*. Wiley: New York, NY, USA, 1998, pp 196–208.
- Shiwei X, Dai X, Meng X, Canhua X, Chaoshuang Ch, Mengxing T et al. Performance evaluation of five types of Ag/AgCl bio-electrodes for cerebral electrical impedance tomography. *Ann Biomed Eng* 2011; **39**: 2059–2067.
- Bolton MP, Ward LC, Khan A, Campbell I, Nightingale P, Dewit O, Elia M. Sources of error in bioimpedance spectroscopy. *Physiol Meas* 1998; **19**: 235–246.
- Bogónez-Franco P, Nescolarde L, Bragós R, Rosell-Ferrer J, Yandiola I. Measurement errors in multifrequency bioelectrical impedance analyzers with and without impedance electrode mismatch. *Physiol Meas* 2009; **30**: 573–587.
- Piccoli A, Rossi B, Pillon L, Bucciantie G. A new method for monitoring body fluid variation by bioimpedance analysis: The RXc graph. *Kidney Int* 1994; **46**: 534–539.
- Piccoli AF for the Italian HD-BIA Study Group. Identification of operational clues to dry weight prescription in hemodialysis using bioimpedance vector analysis. *Kidney Int* 1998; **53**: 1036–1043.
- Pillon L, Piccoli A, Lowrie EG. Vector length as a proxy for the adequacy of ultrafiltration in hemodialysis. *Kidney Int* 2004; **66**: 1266–1271.
- Nescolarde L, Piccoli A, Román A, Núñez A, Morales R, Tamayo J et al. Bioelectrical impedance vector analysis in haemodialysis patients. Relation between oedema and mortality. *Physiol Meas* 2004; **25**: 1271–1280.
- Piccoli A, for the Italian CAPD-BIA study group. Bioelectrical impedance vector distribution in peritoneal dialysis patients with different hydration state. *Kidney Int* 2004; **65**: 1050–1063.
- Nescolarde L, Doñate T, Piccoli A, Rosell-Ferrer J. Comparison of segmental with whole-body impedance measurements in peritoneal dialysis patients. *Med Eng Phys* 2008; **30**: 817–824.
- Piccoli A, Brunani A, Savia G. Discriminating between body fat and fluid changes in the obese adult using bioimpedance vector analysis. *Int J Obesity* 1998; **22**: 97–104.
- Gastelurrutia P, Nescolarde L, Rosell-Ferrer J, Domingo M, Ribas N, Bayes-Genis A. Bioelectrical impedance vector analysis (BIVA) in stable and non stable heart failure patients: a pilot study. *Int J Cardiol* 2010; **146**: 262–264.
- Piccoli A, Codognotto M, Di Pascoli L, Boffo G, Caregaro L. Body mass index and agreement between bioimpedance and anthropometry estimates of body compartments in anorexia nervosa. *J Parenter Enteral Nutr* 2005; **29**: 148–156.
- De Palo T, Messina G, Edefonti A, Perfumo F. Normal values of the bioelectrical impedance vector in childhood and puberty. *Nutrition* 2000; **16**: 417–424.
- Piccoli A, Fanos V, Peruzzi L, Schena S, Pizzini C, Borgione S et al. Reference values of the bioelectrical impedance vector in neonates in the first week after birth. *Nutrition* 2002; **18**: 383–387.

- 26 Margutti AV, Monteiro JP, Camelo JS. Reference distribution of the bioelectrical impedance vector in healthy term newborns. *Br J Nutr* 2010; **104**: 1508–1513.
- 27 L'Abée C, Poorts-Borger PH, EHGN Gorter, Piccoli A, Stolk RP, Sauer PJJ. The bioelectrical impedance vector migration in healthy infants. *Clin Nutr* 2010; **29**: 222–226.
- 28 Nescolarde L, Núñez A, Bogónez-Franco P, Lara A, Vaillant G, Morales R *et al*. Reference values of the bioimpedance vector components in a Caribbean population. *e-SPEN J* 2013; **8**: e141–e144.
- 29 Ward LD, Heitman BL, Craig P, Stroud D, Azinge EC, Jebb S. Association between ethnicity, body mass index, and bioelectrical impedance. *Ann NY Acad Sci* 2001; **258**: 199–202.
- 30 Piccoli A, Pillon L, Dumler F. Impedance vector distribution by sex, race, body mass index, and age in the United States: standard reference intervals as bivariate Z scores. *Nutrition* 2002; **18**: 156–170.
- 31 Bosity-Westphal A, Danielzik S, Dörhöfer RP, Piccoli A, Müller MJ. Patterns of bioelectrical impedance vector distribution by body mass index and age: implications for body-composition analysis. *Am J Clin Nutr* 2005; **82**: 60–68.
- 32 Piccoli A, Nigrelli S, Caberlotto A, Bottazzo S, Rossi B, Pillon L, Maggiore Q. Bivariate normal values of the bioelectrical impedance vector in adult and elderly populations. *Am J Clin Nutr* 1995; **61**: 269–270.
- 33 Norman K, Syobaus N, Pirlich M, Bosity-Westphal A. Bioelectrical phase angle and impedance vector analysis—clinical relevance and applicability of impedance parameters. *Clin Nutr* 2012; **31**: 854–861.
- 34 Camina Martín MA, De Mateo Silleras B, Nescolarde Selva L, Barrera Ortega S, Domínguez Rodríguez L, Redondo del Río MP. Bioimpedance vector analysis and conventional bioimpedance to assess body composition in elderly adults with dementia. *Nutrition* 2015; **31**: 155–159.
- 35 Toso S, Piccoli A, Gusella M, Menon D, Bononi A, Crepaldi G. Altered tissue electric properties in lung cancer patients as detected by bioelectric impedance vector analysis. *Nutrition* 2000; **16**: 120–124.
- 36 Lukaski HC, Piccoli A. Bioelectrical impedance vector analysis for assessment of hydration in physiological states and clinical conditions In: Preedy V (ed), *Handbook of Anthropometry*. Springer: London UK, 2012, pp 287–315.
- 37 Lukaski HC, Bolonchuk WW, Hall CB, Siders WA. Validation of tetrapolar bioelectrical impedance method to assess human body composition. *J Appl Physiol* 1986; **60**: 1327–1332.
- 38 Piccoli A, Pastori G. *BIVA Software*. University of Padova: Padova, Italy, 2002.
- 39 Hotelling H. The generalization of Student's ratio. *Ann Math Stat* 1931; **2**: 360–378.
- 40 Camina Martín MA, de Mateo Silleras B, Redondo del Río MP. Body composition analysis in older adults with dementia. Anthropometry and bioelectrical impedance analysis: a critical review. *Eur J Clin Nutr* 2014; **68**: 1228–1233.
- 41 Ward LC, Dylke E, Czerniec S, Isenring E, Kilbreath SL. Confirmation of the reference impedance ratios used for assessment of breast cancer-related lymphedema by bioelectrical impedance spectroscopy. *Lymphat Res Biol* 2011; **9**: 47–51.
- 42 Boone KG, Holder DS. Effect of skin impedance on image quality and variability in electrical impedance tomography: a model study. *Med Biol Eng Comput* 1996; **34**: 351–354.
- 43 Da Silva TK, Berbigier MC, Rubin Bde A, Moraes RB, Corrêa Souza G, Schweigert Perry ID. Phase angle as a prognostic marker in patients with critical illness. *Nutr Clin Pract* 2015; **30**: 261–265.
- 44 Beberashvili I, Azar A, Sinuani I, Shapiro G, Feldman L, Stav K *et al*. Bioimpedance phase angle predicts muscle function, quality of life and clinical outcome in maintenance hemodialysis patients. *Eur J Clin Nutr* 2014; **68**: 683–689.
- 45 Chumlea WC, Guo SS, Siervogel RM. Phase angle spectrum analysis and body water. *Appl Radiat Isot* 1998; **49**: 489–491.
- 46 Norman K, Stobäus N, Zocher D, Bosity-Westphal A, Szramek A, Scheufele R *et al*. Cutoff percentiles of bioelectrical phase angle predict functionality, quality of life, and mortality in patients with cancer. *Am J Clin Nutr* 2010; **92**: 612–619.
- 47 Böhm A, Heitmann BL. The use of bioelectrical impedance analysis for body composition in epidemiological studies. *Eur J Clin Nutr* 2013; **67**: S79–S85.